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Hyaluronan Accumulation in Wounded Epidermis: A Mediator of Keratinocyte Activation

Raija H. Tammi[1] and Markku I. Tammi[1]

The high-molecular-mass polysaccharide hyaluronan is abundant in the extracellular space between adjacent keratinocytes throughout the vital part of epidermis. It has a rapid turnover, and its content is subject to large fluctuations due to physiological and environmental conditions, with the strongest effects mediated by EGFR signaling. Using an elegant organotypic culture system, Monslow *et al.* (2009, this issue) demonstrate that heparin-binding (HB)-EGF released from its membrane anchor is the major ligand of EGFR in injured epidermis, accounting for the autocrine and paracrine activation of hyaluronan synthesis by the keratinocytes in the neighborhood, thus facilitating the epidermal wound-healing response.

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Organ cultures and organotypic cultures in epidermal research

Isolated keratinocytes in monolayer cultures lack many of the regulatory processes operating in the epidermis, where stratified keratinocytes exist at different stages of differentiation. One example is heparin-binding (HB)-EGF, which is expressed and secreted by differentiating keratinocytes but targets proliferating cells. Another example is hyaluronan, which *in vivo* is restricted to the narrow spaces between keratinocytes and is prevented from diffusing beyond the basal lamina and the water barrier in stratum corneum, but it is free to escape into a vast volume of medium in monolayer cultures. To mimic conditions that keep hyaluronan in its normal domain and concentration, and to provide HB-EGF diffusion routes and spaces similar to those *in vivo*, Monslow *et al*. (2009, this issue) used a culture system based on a unique epidermal cell line (REK) that can form an "epidermis" structurally and functionally resembling its "real" counterpart (Tammi *et al.*, 2000). REK-organotypic cultures do not need dermal fibroblasts to differentiate fully, thus avoiding the complex interplay between the different cell types in other organotypic or explant skin organ culture models. The paper by Monslow *et al*. demonstrates the usefulness of these REK-organotypic cultures in wound-healing research.

Hyaluronan is involved in tissue remodeling

Haluronan (or hyaluronic acid or hyaluronate) is abundant in embryonic tissues, reaching peak concentrations in each organ before maturation, when cells proliferate and migrate to their final positions (Toole, 2004). The same pattern of transient hyaluronan accumulation is seen whenever adult tissues undergo rapid remodeling, whether as a result of injury, inflammation, or cancer. Hyaluronan is deposited around inflamed joints in rheumatoid arthritis, causing morning stiffness, and it promotes tumor growth and invasion when it accumulates on malignant cells or in the surrounding matrix. In each case, it forms a pliable, temporary matrix that enables cell proliferation or movement, and it amplifies growth factor signals important in the remodeling process. Transient accumulation of hyaluronan in granulation tissue, a model of dermal wound response, has been known for decades, and the prenatal abundance of hyaluronan has been suggested to play a role in scarless wound healing of fetal skin (Mack *et al.*, 2003). Recent work shows that activation of hyaluronan synthesis also occurs in wounded adult epidermis (Maytin *et al.*, 2004; Tammi *et al.*, 2005).

Hyaluronan in the epidermis

Hyaluronan fills the narrow spaces between human epidermal basal and spinous cells (Tammi *et al.*, 1988). Its concentration in this space has been calculated to be as high as 2.5 mg/ml, comparable to its concentration in synovial fluid. Following trauma *in vivo*, the concentration of epidermal hyaluronan rapidly increases up to sevenfold (Tammi *et al.*, 2005). This response requires only compromised epidermal barrier function, without direct impact on the dermis (Maytin *et al.*, 2004; Tammi *et al.*, 2005). The hyaluronan response is not restricted to the area immediately adjacent to the wound, but spreads considerably from wound margins, indicating the release of factors capable of activating hyaluronan synthesis (Figure 1 and Tammi *et al.*, 2005).

In the basal state, the half-life of hyaluronan is only about one day in human skin organ cultures (Tammi *et al.*, 1991) and even shorter in the organotypic epidermis of rat keratinocytes (REK; Tammi *et al.*, 2000). The biological relevance of this short turnover

[1]*Department of Biomedicine, Biocenter Kuopio, University of Kuopio, Kuopio, Finland*

Correspondence: Dr Raija H. Tammi, Department of Biomedicine and Anatomy, University of Kuopio, P.O. Box 1627, Kuopio, Finland. E-mail: rtammi@uku.fi

time has been puzzling, but it becomes more understandable in light of the work of Monslow *et al*. (2009), which illustrates the extremely rapid upregulation of epidermal hyaluronan synthesis following mechanical injury. The new data emphasize and corroborate earlier *in vivo* work (Maytin *et al*., 2004; Tammi *et al*., 2005), stressing the urgent need to rebuild a disrupted epidermal barrier by keratinocytes. But how is the rapid hyaluronan surge involved in this process?

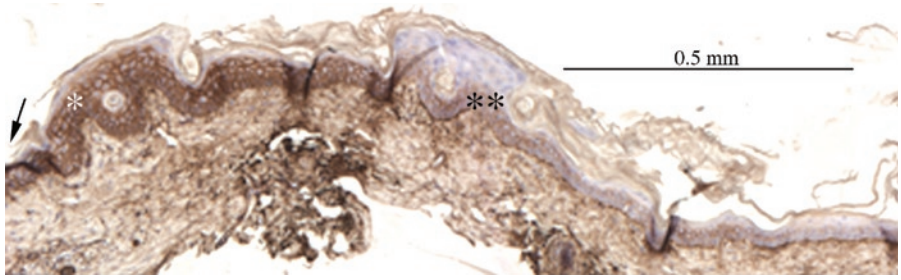

Figure 1. Spreading of the hyaluronan response in injured epidermis. Mouse tail skin was processed for histology 3 days after wounding by tape stripping and stained using a biotinylated hyaluronan binding probe as previously described (Tammi *et al.*, 2005). The brown color indicates the localization of hyaluronan. The images were taken laterally from the wound edge (indicated by the arrow). Hyaluronan staining in the epidermis close to the wound (white asterisk) is stronger than the more lateral signal; however, the increased hyaluronan reaction is still clearly visible 1 mm from the edge (black asterisks) compared with the rather faint staining closer to the right side of the image.

Epidermal activation and re-epithelialization after wounding

Keratinocytes from the basal and spinous cell layers adjacent to a wound acquire an activated phenotype (Freedberg *et al.*, 2001), in which proliferation is increased and differentiation is retarded, forming a pool of migration-competent keratinocytes to rapidly cover the wound and re-establish the permeability barrier. Growth factor receptors and their ligands originating from epidermis (like HB-EGF), dermis (like keratinocyte growth factor (KGF)), and inflammatory cells have been shown to be important in epidermal activation and wound healing (reviewed by Barrientos *et al*., 2008). The culture model used by Monslow *et al*. (2009) clarifies an important point in this process: Growth factor signals from epidermis alone (HB-EGF) suffice to stimulate widespread hyaluronan synthesis as a part of the wound healing response. Although KGF and other mediators from the dermal side contribute to and tune the healing response, these factors do not dominate (at least in the autocrine and paracrine process of keratinocyte hyaluronan synthesis).

> Hyaluronan is central to epidermal repair and maintenance.

An idea supported by the present work is that hyaluronan is an important contributor to re-epithelialization. Data are accumulating to support this idea, as described below. Factors that stimulate hyaluronan synthesis (e.g., EGF, KGF, and retinoic acid) also increase proliferation and epidermal thickness, whereas factors that decrease hyaluronan synthesis (e.g., transforming growth factor-β and glucocorticoids) inhibit proliferation and promote differentiation (Karvinen *et al*., 2003; Pasonen-Seppänen *et al*., 2003, 2008; Pienimäki *et al*., 2001; Ågren *et al*., 1995). Accordingly, when hyaluronan content is reduced by the hyaluronan synthesis inhibitor 4-methylumbelliferone (Rilla *et al*., 2004), epidermal activation by EGF is blocked, and when hyaluronan is removed by hyaluronidase treatment (Passi *et al*., 2004), epidermal differentiation is enhanced. These findings suggest that an increase in epidermal hyaluronan content supports keratinocyte proliferation and puts the brakes on terminal differentiation, providing maximum numbers of activated keratinocytes for resurfacing an open wound. Hyaluronan bulging in the intercellular spaces reduces cohesion between keratinocytes, making room for cell movement and inducing cytoplasmic signals such as Rac1, which enhance cell migration (Bakkers *et al*., 2004) and the formation of filopodia/microvilli (Kultti *et al*., 2006; Rilla *et al*., 2008).

Future lines of research and unanswered questions

The literature supports the hypothesis that stimulation by hyaluronan supports a migratory and proliferative phenotype of epidermal keratinocytes. However, it has not been clearly determined whether the hyaluronan must be synthesized by the keratinocytes themselves or whether hyaluronan provided exogenously could serve the same purpose. More direct data on hyaluronan in epidermal physiology might arise from experiments in which epidermal hyaluronan synthesis is completely inhibited. Currently, deletion of the *has2* gene, the isoenzyme of the hyaluronan synthase (*has*) family with the highest synthetic capacity, causes embryonal lethality, whereas *has1* and *has3* knockouts have no phenotype (Camenisch *et al*., 2000). Indeed, future studies are needed to specifically target *has2* expression in epidermis.

Related to the importance of *Has* expression in wound healing is the role of cell surface receptors such as CD44 and TLR2 (Jiang *et al*., 2007), which have been reported to inform cells about the size and content of hyaluronan at the cell surface and to mediate the effects of hyaluronan. However, the addition of exogenous hyaluronan often does not recapitulate the effects of endogenous hyaluronan synthesis, and CD44 knockout mice have very minor skin problems, indicating that cell surface receptors cause only a part of the response.

Interesting new questions about *has* expression and function have arisen from this study. One relates to the rapid increase in hyaluronan synthesis following injury. Although *has2* belongs to the primary target genes of EGFR signaling (Saavalainen *et al*., 2005)

and *has* gene expression is rapidly upregulated by HB-EGF, augmented hyaluronan production appears too rapidly (15 min) to be accounted for by transcriptional upregulation. Does existing Has protein on plasma membrane become activated, for instance, by phosphorylation (Goentzel *et al*., 2006), or are Has proteins transferred from their ER/Golgi stores to the plasma membrane, the only cellular site where Has appears to be enzymatically active (Rilla *et al*., 2005)?

Can epidermal hyaluronan become a therapeutic target?

It can be envisioned that diseases associated with excessive epidermal activation might benefit from suppression of hyaluronan synthesis, for instance, by local application of currently known (Jokela *et al.,* 2008; Rilla *et al.,* 2004) or new inhibitors. On the other hand, opposite clinical indications might arise for treating atrophic epidermis or enhancing wound healing responses. Hyaluronan is already being applied to wounds, dressings, and cosmetic preparations alone or in combination with skin cells or growth factors (Price *et al.,* 2007), even though the molecular mechanisms of its effects remain obscure. Future treatment options depend on whether exogenously applied hyaluronan is found to retain the qualities associated with endogenously synthesized hyaluronan.

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